Science, 282:293-296, 1998. Muteins can comprise amino acid substitutions, insertions, and deletions, including replacing naturally-occurring amino acids with non-naturally occurring amino acids. Amino acid substitution can be made by replacing one homologous amino acid for another. Homologous amino acids can be defined based on the size of the side chain and degree of polarization, including, small nonpolar: cysteine, proline, alanine, threonine; small polar: serine, glycine, aspartate, asparagine; large polar: glutamate, glutamine, lysine, arginine; intermediate polarity: tyrosine, histidine, tryptophan; large nonpolar: phenylalanine, methionine, leucine, isoleucine, valine. Homologous acids can also be grouped as follows: uncharged polar R groups, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine; acidic amino acids (negatively charged), aspartic acid and glutamic acid; basic amino acids (positively charged), lysine, arginine, histidine. Homologous amino acids also include those described by Dayhoff in the Atlas of Protein Sequence and Structure 5, 1978, and by Argos in EMBO J., 8, 779-785, 1989. Homologous amino acid replacement or modification can be utilized when it is desired to maintain, or enhance a PI3Kγ activity. Non-homologous amino acid replacement or modification can be utilized when it is desired to destroy or decrease a PI3Kγ activity. A polypeptide mutein, and its corresponding nucleotide coding sequence, can have an amino acid sequence as set forth in Fig. 3 except where one or more positions are substituted by homologous amino acids, e.g., where there are 1, 5, 10, 15, or 20 substitutions. Amino acid substitutions can also be made based on analogy to related other PI3Ks.

A PI3Kγ of the present invention, fragments, and muteins thereof, can also comprise various modifications, where such modifications include lipid modification, methylation, phosphorylation, glycosylation, covalent modifications (e.g., of a side chain of an amino acid). Modifications to the polypeptide can be accomplished according to various methods, including recombinant, synthetic, chemical, etc.

The present invention also relates to antibodies which are "specific-for" a particular polypeptide comprising a defined amino acid sequence of a PI3Kγ. The phrase "specific-for" indicates that the antibody is selective for the defined amino acid sequence. The amino acids sequences can possess other immunogenic activities, as well, e.g., stimulating of T-cells, macrophages, B-cells, dendritic cells, etc. These responses can be measured routinely.

An aspect of the present invention relates to polypeptides, fragments and muteins, of PI3Ks that possess phospholipid binding, and PI3Ks which display modified phospholipid binding activity. As mentioned, PI3Ks phosphorylate phosphoinositides, and analogs and derivatives thereof, at a 3-hydroxyl group. The catalytic reaction involves binding of a phospholipid substrate to the enzyme. A polypeptide fragment of PI3Kγ has been identified which possesses phospholipid activity. This fragment can also be referred to a phospholipid binding domain to indicate its primary activity. By the term "fragment," it is meant any sequence of amino acids which is less than the full-length size of a PI3K. A PI3K phospholipid binding domain, preferably consists essentially of a C-terminal helix kα12, catalytic loop, activation loop, and amino acid residues Lys807, Lys808, Arg947, and Lys973. The catalytic loop preferably consists essentially of amino acids 943-951 and the activation loop preferably consists essentially of amino acids 964-988, and kα12 preferably consists essentially of amino acids 1081-1090.

A PI3K mutein, or polypeptide fragment thereof, which possesses phospholipid binding activity preferably comprises Lys807, Lys808, Arg947, and Lys973. Muteins which possess less than normal phospholipid binding activity preferably comprise amino acid substitutions at one or more positions Lys807, Lys808, Arg947, and Lys973. Phospholipid binding to PI3K can be measured conventionally, e.g., using radiolabeled phospholipids.

By the phrase "less than normal binding activity," it is meant that such mutein (full-length PI3K or a fragment thereof), displays an amount of activity which is reduced when compared to the wild-type, not mutated, enzyme. Such amount can be reduced by any quantity, e.g., 5%, 10%, 25%, 50%, or even a total loss of activity. Phospholipid binding activity can be measured by any effective method.

An isolated polypeptide mutein of PI3K can comprise a phospholipid binding domain, which domain comprises the C-terminal helix kα12, catalytic loop, and activation loop sequences of Fig. 3, and at least 95% sequence identity to the remaining sequence in Fig. 3. In general, the phrase that a domain, region, etc., comprises a sequence of Fig. 3, it meant that the polypeptide has 100% sequence identity to the sequence disclosed in Fig. 3. For this mutein, the phospholipid domain has 100% sequence identity to its sequence in Fig. 3, but the remaining regions have

less 100% sequence identity, such as 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, but less than 100%. Such muteins can lack all known catalytic activities of PI3K, but still possess the phospholipid binding activity.

The present invention also relates to methods of modulating phospholipid binding activity, e.g., binding of a phospholipid substrate to a PI3K enzyme, fragment, mutein, etc. Modulating can be accomplished in any manner, e.g., modifying the amino acid sequence of PI3K, contacting an active site or region with a modifying agent, e.g., a chemical agent which modifies the chemical groups, contacting an active site or region with a ligand, e.g., an antibody. In particular, as mentioned above, regions involved in phospholipid binding, comprise a C-terminal helix kα12, catalytic loop, and activation loop. The catalytic loop preferably consists essentially of amino acids 943-951 and the activation loop preferably consists essentially of amino acids 964-988. Modification of these regions, particularly amino acids Lys807, Lys808, Arg947, and Lys973, can affect substrate binding activity.

Another aspect of the present invention relates methods of modulating lipid kinase catalysis. Inhibition can be accomplished by various methods, including, e.g., modifying the amino acid sequence of a PI3K, contacting an active site, or amino acid residue thereof, with a modifying agent, etc. For example, the histidine at amino acid 968 has been identified as involved in the deprotonation of the 3-hydroxy of the lipid headgroup. Alteration of the histidine would be expected to effect the enzyme's activity, e.g., by inhibiting, blocking, decreasing, reducing, enhancing, increasing, etc., its activity. Non-conservative (non-homologous) amino acid substitution can be expected to reduce catalytic activity. Conservative (homologous) amino acid substitution can be expected to not affect, or to increase catalytic activity.

Another way of modulating the lipid kinase activity is to modify the amino acid sequence of a produced PI3K, e.g., at its active site, such as the amino acid residues surrounding and including His968. Any agent which can chemically modify an amino acid can be used, including, oxidizing agents, reducing agents, alkylating agents, etc. In addition, ligands which attach to the active site can be used, such as substrate analogs, antibodies, e.g., an antibody which recognizes an amino acid sequence comprising His968.